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Taking Root: An Analysis of the Best Method for Active Forest Regeneration in the Cloud Forest

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Abstract

The cloud forest is a biodiversity hotspot and provides many vital ecological and economic services. Unfortunately, deforestation for pastures in the lower montane cloud forest is high. Many pastures become abandoned, leaving highly disturbed open areas of land where natural forest regeneration is poor. Human intervention to encourage ecological restoration is very important to re-establish the proper structure and functioning of the ecosystem. Forest regeneration projects are rare and often unsuccessful, which is why increased knowledge and skills of successful and practical methods for assisted regeneration are needed. The Inti Llacta Reserve in the Pichincha province in Ecuador protects a biodiverse area of cloud forest and is dedicated to sustainable land practices, including restoration of retired pasture. This study compares different restoration methods on the reserve to determine the most successful treatment to facilitate natural forest regeneration. Four different areas of retied pasture undergoing early secondary succession were surveyed: an active experiment with grass eradication where key pioneer, coloniser, and climax species were planted; an area where Aliso trees were planted five years ago; an area where *Tithonia* bushes were planted seven years ago, and an area of passive regeneration. Successful regeneration was expected to occur in areas with low invasive grass cover and high seed rain. The results show that the active experiment had the highest density of regenerated species despite the highest grass cover. Seed input from established plants or from the forest was an important factor for success in this site. Shade was most successful in reducing grass cover but natural regeneration was slow, so another type of intervention to reduce grass cover is needed to speed up regeneration.

Resumen

El bosque nublado es un área lleno de biodiversidad y provee muchos servicios vitales para la ecología y la economía. Desafortunadamente, la deforestación para las pasturas en el bosque nublado es alta. Muchas pasturas son abandonadas, dejando áreas abiertas de la tierra muy degradadas en donde la regeneración natural del bosque es pobre. La intervención humana para estimular la restauración ecológica es muy importante para restablecer la estructura y función correcta del ecosistema. Proyectos del regeneración del bosque son raros y a menudo no tienen éxito, y por eso es necesario aumentar el saber y las habilidades de métodos éxitos y prácticos para la regeneración asistida. La Reserva Inti Llacta en la provincia Pichincha en Ecuador protege un área del bosque nublado biodiversa y está dedicando a los prácticos sustentables, incluyendo la restauración de las pasturas retiradas. Este estudio compara diferentes métodos de restauración en la reserva para determinar el método más exitoso para facilitar la regeneración natural del bosque. Cuatro áreas diferentes de pastura retirada en proceso del inicio de sucesión secundaria estaban evaluados: un experimento activo con erradicación del pasto y especies claves sembradas de tipo pionera, colonizador, y clímax; un área con alisos sembrados hace cinco años; un área con arbustos de *Tithonia* sembrados hace siete años, y un área de regeneración pasiva. Había supuesto que regeneración exitosa ocurría en áreas bajas en cobertura de pasto y altas en la lluvia de semillas. Los resultados muestran que el experimento activo tenía la densidad de especies regeneradas más alta a pesar de tener la cobertura de pasto más alta. La contribución de

semillas de las plantas establecidas o del bosque fue un factor importante para el éxito de regeneración en este sitio. La sombra tenía el más éxito en reducir la cobertura de pasto, pero la regeneración natural fue lenta, entonces otro tipo de intervención es necesario para reducir la cobertura de pasto y apurar la regeneración.

Topic Codes: 608, 613, 614

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Introduction

The cloud forest, also known as the lower montane forest, occurs between 700 – 2500m on both sides of the Andes mountain range. The cloud forest arguably has the highest beta diversity in the world and is an important hotspot for biodiversity. This region receives high precipitation and humidity, resulting in high density and diversity of epiphytes and mosses (Policha, 2008). The vegetation and ground hold a lot of water from horizontal rain, playing an important role in watershed hydrology (Ataroff & Rada, 2000). Maintaining the diversity and density of vegetation in the cloud forest is vital due to the many ecological and economic services it provides. These services include providing habitat for thousands of species, above and below ground carbon sequestration, water catchment, prevention of nutrient leaching, and protection against erosion (Silver, 2001). Many plants found in the cloud forest hold medicinal uses that are widely used in folk medicine. Roughly 25-40% of plants in Ecuador have medicinal value, used by around 80% of people living in the humid tropics, who rely on traditional medicine derived from plants to treat their healthcare needs (Policha, 2008). There is great potential to discover additional medicinal uses that are currently unknown.

Despite its undeniable value, the cloud forest is under threat from human activities such as deforestation for urban expansion, agriculture, logging, and mining. Conservation is limited due to lack of regulation and poor land-management practices. As a result, pristine areas of intact primary forest are being destroyed at an alarming rate. The cloud forest has one of the highest deforestation rates of any tropical forest globally, losing 1.1% of primary forests to deforestation annually (Bussman R. W.), with around 44% of the original global cover gone (Avendaño-Yanez, et al, 2014). In the area between Venezuela and Bolivia, only 10% of original forest remains (Báez & Ambrose, 2010). The main cause of deforestation is clear-cutting of land for pastures. Unfortunately, due to decline in land productivity or changing economic pressures, many pastures are abandoned within a few years and remain unused, and natural regeneration of these highly disturbed areas is poor (Holl, 2006).

Natural forest regeneration occurs frequently when a gap opens up in the forest, caused by disturbances such as high winds or storms that cause trees to fall. When a gap opens up in the forest canopy, the forest regenerates from seeds in the native seed bank or from seed rain, which is the dispersal of seeds by wind, birds, animals, or water. Succession starts with pioneer species that grow fast and are resistant to harsh conditions and need direct sunlight to germinate. The pioneer species then provide shade to allow shade-tolerant species to grow. These climax species are slow-growing and less resistant to harsh conditions. They have a long life-span, and eventually out-compete the pioneer species and come to dominate the canopy. Pioneer species indicate an area of disturbed forest in early succession, whereas climax species indicate an area of late succession or primary forest (Corlett, 1995). However, it takes many decades for the species composition of secondary forest to return to that of primary forest (Guariguata & Ostertag, 2001). Natural regeneration will occur in any disturbed area unless there are limiting factors that impede it.

In highly disturbed areas like abandoned pastures, there are many factors that inhibit natural regeneration, and so human intervention must aim to reduce or eliminate these factors in order to facilitate natural regeneration. Proper ecological restoration is vital in these areas to combat habitat loss and species extinction, increase biodiversity, and restore the important services that the forest provides. Ecological restoration aims to re-establish the entire ecosystem, so that it functions properly, with both abiotic and biotic factors intact, just like natural succession. One factor that limits regeneration in pastures is low seed input. Seeds germinate naturally from the seed bank or are deposited by seed rain. Seed rain is low in areas that are far from the forest because fewer seeds are dispersed over great distances. Seed rain in pastures is also low because seed dispersers tend to avoid open areas that have high exposure and a high risk of predation (Bol & Vroomen, 2008). Forest seeds lose viability quickly, and so regeneration is dependent on recently dispersed seeds (Holl, 2006). Therefore, a method to encourage natural regeneration in open areas is to introduce seeds or seedlings of key species. But even when seeds are present, plants may not establish due to intense competition with invasive species. Invasive grasses and herbs tend to dominate abandoned pastures because they can withstand harsh conditions such as drought, direct sunlight, and poor soil quality. Grass removal is another component to encourage natural regeneration to allow seeds to access adequate water, nutrients, and space. Complete grass removal either by hand or with a machine is an option, but it is very labor-intensive and may not be practical. Another option is to plant pioneer species that will grow quickly and create shade under which shade-intolerant grass cannot grow. By reducing the grass coverage, secondary and climax species will be able to grow in the shade without intense competition. Recovery is also limited by abiotic factors such as low soil nutrients, soil compaction, seasonal drought, lack of water availability, and poor microclimate (Bol & Vroomen, 2008). Soil compaction by cattle can affect root growth, gas exchange, infiltration rates, and seed germination (Holl, 2006). A way to combat these factors is to plant species that improve the quality of the soil. Nitrogen-fixing trees are a valuable source of essential nutrients for the soil. Planting trees will also add to the organic matter in the soil through litter fall, and help to decrease erosion and water runoff.

Avendaño-Yanez et al (2014) found that early successional and colonizer species can facilitate growth and success of intermediate and late successional species in areas of disturbed tropical cloud forest. These species are ecologically beneficial because they are fast-growing, highly resistant, and contribute to the amount of organic matter in the soil. In contrast, intermediate and late successional species are less resistant to extreme conditions, such as direct sunlight, exposure to wind, water stress and high herbivory that occur in disturbed areas, and so do not establish successfully in these areas. However, in the presence of early successional species that mitigate these harsh conditions, there is more potential for establishment of intermediate and late successional species. The early successional species provide shade which increases the growth and survival of intermediate and late successional species, and can also act to reduce the number of competitive herb species (Avendaño-Yanez et al, 2014). Bol & Vroomen (2008) explain that planted trees provide a diversity of micro-climates to facilitate the growth of both shade-intolerant and shade-tolerant species. Furthermore, fruiting or flowering trees are beneficial because they attract seed dispersers, leading to increased seed input (Bol & Vroomen, 2008).

Forest regeneration not only has ecological benefits but socio-economic benefits as well. Restoring ecosystems can combat poverty, malnutrition, and migration caused by bad land-management practices and deforestation (Báez & Ambrose, 2010). Large-scale restoration projects are needed, but not always feasible because communities live on the land and farmers depend on their crops for survival. Substantial compensation would need to be provided for the economic losses that would result, which is rarely available. Small-scale restoration is much more feasible, practical, and achievable in the short-term (Báez & Ambrose, 2010). For long-term success, it is vital to directly involve the community in restoration projects (Vargas Ríos, 2011). Successful projects also include environmental education to increase knowledge about the values of the ecosystem, and revision or improvement of agricultural practices to use more sustainable methods or require less land. Examples of alternative, sustainable agriculture include agro-forestry or silvo-pastoral systems (Huxley, 1999).

Responsible and effective ecological restoration of abandoned pastures in the cloud forest is clearly needed. However, the most successful technique to assist regeneration in the cloud forest is not well studied. This study evaluates different methods of human intervention on abandoned pastures to determine the most successful method to mitigate the limiting factors of regeneration. It was predicted that grass removal would encourage regeneration by reducing competition. Shaded areas were predicted to have the least grass cover. Grass removed with root was predicted to be more successful in reducing grass cover than superficial grass removal. It was also predicted that planted trees would facilitate regeneration by improving the microclimate for other species to germinate and by adding to the seed bank. It was thought that planting trees at a high density and diversity would produce the highest density and diversity of regenerated species. Areas that were closest to the edge of the forest were predicted to have the highest diversity of regenerated species because of the greater input of seeds from seed rain. Passive regeneration with no human intervention was predicted to have the lowest density and diversity of regenerated species because of low seed input and poor conditions for germination.

Study Area

The study took place in Inti Llacta reserve, near the town of Nanegalito in the Pichincha province. The reserve is located in the northwest cloud forest of the Choco Bioregion in Ecuador, at an altitude of 1700-1900m. The reserve was founded in 1984 and covers 88 hectares. The reserve is dedicated to conservation, sustainable tourism, and organic agriculture. This study was part of a long-term project looking at ecological restoration on the reserve. Four different sites of abandoned pastureland were compared to assess different types of forest regeneration and determine the most successful intervention method.

Site one: Active experiment

The first site became an abandoned pasture ten years ago, and is now undergoing an experiment in assisted ecological regeneration. Four different treatments were applied in January, 2015 to facilitate secondary succession and are being monitored to determine the most successful treatment. The first two treatments compare high and low levels of density and diversity of established plants. The other two treatments compare complete or superficial eradication of grass. The treatments are in four different plots, measuring 7x13m each. Plot one is eradication of grass and root with simple density and diversity of plants; plot two is eradication of grass and root with dense and diverse plants; plot three is superficial eradication of grass with simple density and diversity of plants; and plot four is superficial eradication of grass with dense and diverse plants. The simple treatments have 16 species of colonizer, pioneer, and secondary trees planted with naked roots, with 0.92 individuals/m^2. Resprouting *Tithonia diversifolia* sticks were planted every 2m in between the rows of plants. The dense and diverse treatments had 29 species of colonizer, pioneer, secondary, palm, and climax trees planted with naked roots, with 2.89 individuals/m^2. In all plots, seeds of 5 tree species were sewn, two handfuls in each corner of the plot, to mimic seed rain (see plot designs in appendix). The seedlings and seeds were collected from the surrounding forest. There was also a control plot measuring 9x6m with no treatment. The area is bordered by several mature tree species, including Ortigillo (*Utrica sp*.), Guaba (*Inga sp*.), Cordoncillo (*Piper sp*.), 2 species of *Croton*, Moquillo (*Saurauia sp*.), Canelo (*Ocotea sp*.), *Lauraceae*, Chilco (*Baccharis sp*.), Solano (*Solanum sp*.), Cascarillo (*Cinchona sp*.), and Alisos (*Alnus acuminata*) (see appendix for description of all species). Some armadillo activity was observed in the plots and hole digging disturbed some areas. Inside plot three is a Guayaba tree (*Psidium guajava*), and inside plot four is a Moquillo tree (*Saurauia sp*.), both providing shade. All other areas are completely open and receive direct sunlight. The site had a slight inclination of 5°.

Site two: Aliso trees

The second site is a retired pasture with Aliso trees (*Alnus nepalensis*) planted in 2010. The trees are approximately 8.4m tall and 5m apart. The trees provide shade, with sunlight penetrating through 13.25% of the canopy. The method used to encourage regeneration is to plant a pioneer species to facilitate the growth of other species. The trees provide shade which decreases the growth of competitive grasses, and the roots fix nitrogen which adds important nutrients to the soil. The site is directly next to an active cow pasture. The cows occasionally enter the site and eat the remnant grass, which helps to decrease the grass cover, but may also cause soil compaction and damage to seeds and seedlings. The site is 30.9m from the forest edge. Trees surrounding the site include Ortigillos (*Urtica sp*.), *Ficus sp*., Lecheros (*Sapium sp*.), Canelo (*Ocotea sp*.), Cautillo (*Sapium sp*.), Espino santo (*Berberis sp*.), and Chilco (*Baccharis sp*.). Two plots each measuring 7x13m were established in a flat area.

Site three: *Tithonia diversifolia* bushes

The third site is a retired pasture covered with *Tithonia diversifolia* bushes that were planted in 2008. The bushes are approximately 3.7m tall. Light penetrates through 13% of canopy and the rest is in shade. *Tithonia diversifolia* is a fast-growing shrub that provides shade to inhibit the growth of invasive grasses. It also acts as a natural fertilizer by adding important nutrients to the soil. The site is 39m from the forest edge. Trees within the site include *Miconia sp*., Lechero (*Sapium sp*.), and Ortigillo (*Urtica sp*.). Trees surrounding the site include Espino santo (*Berberis sp*.), Teniche (unidentified species), Cujaco (*Solanum sp*.), Chilco (*Baccharis sp.*), Alisos (*Alnus nepalensis)*, and *Clusia sp*. The site had an inclination of 40-55°. Two plots each measuring 7x13m were established.

Site four: Passive regeneration

The forth site is a retired pasture undergoing natural succession with no human intervention. It was used as a baseline to assess passive regeneration. The site is 25m from the forest edge. Trees within the site include *Piper sp*., Ortigillos (*Urtica sp*.), Palmitos (*Prestoea acuminate)*, and Guaba (*Inga sp*.). Trees surrounding the site include Camacho (*Xanthosoma sp*.), *Asteraceae*, Canelo (*Ocotea sp*.), Juan negro *(Piptocoma discolor)*, Espino santo (*Berberis sp*.), *Ficus sp*., and Pera (unidentified species). The area was shaded, with light penetrating through 17.5% of the canopy. Two plots each measuring 7x13m were established in a flat area.

Setaria Sphacelata was the main invasive grass species in all areas.

Methods

All plots were surveyed for vegetation using a 1x1m PVC pipe quadrant. The quadrant was placed 9 times within the plot, 1.0-1.5m from the border. All areas of the plots were evenly sampled by placing the quadrant 3 times at the lower end, middle, and top of each plot. After carefully placing each quadrant over the vegetation, grass cover, herb cover, and *Tithonia* cover was visually estimated using a grid of 20x20cm sub-quadrants, secured with string inside the larger quadrant. The number of sub-quadrants covered by each type of plant was estimated and then percent coverage was calculated. (Note: in plot one of the first site, where there was dense *Tithonia* regeneration, the size of sub-quadrants was increased to 50x50cm to allow the large plants to enter.) The number of herb species in the quadrant was counted, distinguished based on visible physical differences and field experience. All naturally regenerating tree species were identified to the highest known classification and the number of individuals of each species in the quadrant was counted. Planted tree species were recognized and only trees that had germinated from seeds were counted. (Note: In site one, Ortigillo (*Urtica sp.*) was counted as a herb due to high abundance and density of small seedlings in order to avoid misidentification and skewed data.0

Data was taken to characterize each site. Canopy cover was estimated by taking a photo in the middle of each plot looking straight up at the canopy. A 5x4 grid was superimposed over each photo and the amount of light in each square was estimated to determine the total amount of light entering each plot. For each site, two photos were analyzed (one for each plot), and the average was calculated. Inclination of each site was visually estimated. Types of trees within and surrounding each site were identified. The shortest straight-line distance from the edge of each site to the forest edge was measured using a tape measure. The average height of Aliso trees in site two and *Tithonia diversifolia* bushes in site three were visually estimated.

Data from all four sites was analyzed and compared. Plots two and four were chosen to represent site one because they had dense and diverse treatments, and so were most representative of the active experiment. The habitat of each site was compared by calculating average grass cover, average herb cover, combined grass and herb cover, average number of herb species, average number and density of regenerating tree species, amount of light, and distance from the forest edge. Correlation and linear regression was calculated with Vassarstats to determine significant correlations.

The species richness and diversity was compared across the four sites. First, completeness for each site was estimated with the equation $1-f_0/n$, where f_0 is number singletons in the sample, and n is the total number of individuals sampled. Alpha diversity was divided by completeness to give corrected richness for each site. Simpson's Index and Shannon-Wiener Index were calculated to measure diversity of each site. Simpson's Index was calculated with the equation: $S=\sum[n_i(n_i-1)/N(N-1)]$, where n_i=number of individuals for i^{th} species, and N=total number of individuals. The inverse was taken to produce the effective number of species (Joust). Shannon's Index was calculated by the equation: $H=-\sum[(Pi)*Ln(Pi)]$, where Pi Sp_1 = relative abundance Sp_1. Exponential Shannon's was calculated to get the effective number of species (Joust). Evenness was calculated by the equation: E=H/Hmax, where Hmax=lnS and S=number of different species.

The importance value index (IVI) of each species in each site was calculated by the following equation: IVI = Relative Density (RD%) + Relative Frequency (RF%), where Density (D) Sp_1 = average number individuals $Sp_1/1m^2$, RD% Sp_1 = D Sp_1/total D all species, Frequency (F) Sp_1 = number plots Sp_1 occurs/total number plots examined, and RF% Sp_1 = F Sp_1/total F all species.

Beta diversity was calculated with three different indices to assess the similarity between sites. Sorensen's Index was calculated by the equation: SI=(2C/(A+B))*100, and

Jaccard's Index was calculated by the equation: JI = (C/(A+B-C))*100, where A= number of species in site A, B=number of species in site B, and C=number of common species to both sites. The Morisita-Horn Index was calculated by the equation: MI = [2∑(DNi*ENi)/(da+db)aN*bN]*100, where aN=number of individuals in site A, bN=number of individuals in site B, DNi=number of individuals for i^{th} species in site A, ENi= number of individuals for i^{th} species in site B, da=$\sum DNi^2/aN^2$, and db=$\sum ENi^2/bN^2$ (Mostacedo & Fredericksen, 2000).

Species accumulation curves were created using EstimateS to analyze the completeness and diversity of each sample (Colwell, 2013). The data was extrapolated twice with 100 randomizations to create a smooth curve with upper and lower bounds.

Finally, the data from the four plots in site one were analyzed and compared to determine the most successful treatment in the active experiment. Average grass cover, average herb cover, combined grass and herb cover, average *Tithonia* cover, average number of herb species, and average number and density of regenerating tree species were compared in each plot. Correlation and linear regression was calculated with Vassarstats to determine significant relationships.

The results will reveal the site with the most successful regeneration and the best method of intervention. This knowledge will benefit the reserve in future restoration projects, and contribute to scientific knowledge about how to restore disturbed areas of cloud forest in the most successful way.

Results

Habitat comparisons

Site one had the most grass coverage, about 4.6 times as much as site four, which had the lowest grass coverage. Site four had the most herb coverage, about 5.2 times as much as site two, which had the least herb coverage. Site one had the highest coverage of grass and herbs combined, about 4.2 times as much as site two, which had the least grass and herb coverage. Site one had about 6.4 more herb species on average than site four. Site three had the highest number of regenerating tree species, five more species on average than site four, which had the least. Site one had the highest density of regenerating tree species, about 5.1 times the density of site four. Site one had direct sunlight, about 7.7 times as much light as site three which had the least amount of light. Site one bordered the forest edge whereas site three was the furthest away from the forest edge (Table 1).

Table 1. Habitat descriptions of all sites (mean, n=18 for coverage, number of herb species, and density of regenerating species, n=2 for % light).

Study Site	% Grass	% Herb	% Grass and Herb	# Herb Species	Total # regenerating species	Density regenerating species (#ind./m^2)	% Light	Distance from forest edge (m)

One	32.04	20.80	52.84	8.3	9	5.6	100.00	0.0
Two	7.87	4.78	12.65	2.6	10	2.3	13.25	30.9
Three	10.67	9.82	20.49	3.4	12	4.9	13.00	39.0
Four	6.93	25.04	31.97	1.9	7	1.1	17.50	25.0

There was a significant positive correlation between grass cover and amount of light in all sites (correlation and linear regression, p=0.0074; Figure 1).

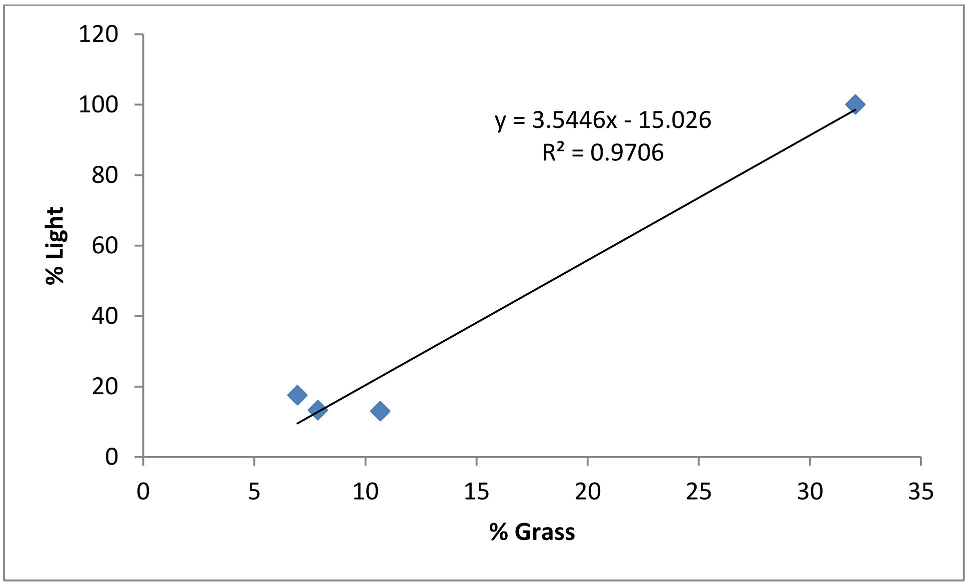

Figure 1. The relationship between grass cover and amount of light for all sites (mean, n=2 for light, n=18 for grass cover).

There was a significant positive correlation between grass cover and number of herb species (correlation and linear regression, p=0.002; Figure 2).

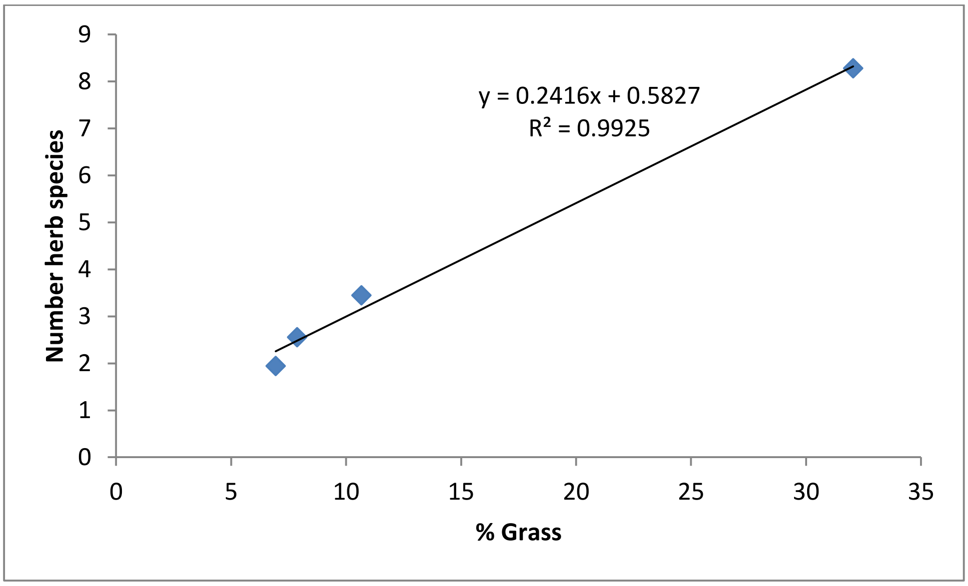

Figure 2. Grass coverage vs. number of herb species for all sites (mean, n=18).

The number of regenerating tree species tended to decrease with increasing grass and herb coverage, but the relationship was not significant. However, there was a significant positive correlation between density of regenerating species and grass cover for sites two, three, and four (correlation and liner regression, p=0.0289; Figure 3). No other significant relationships were found.

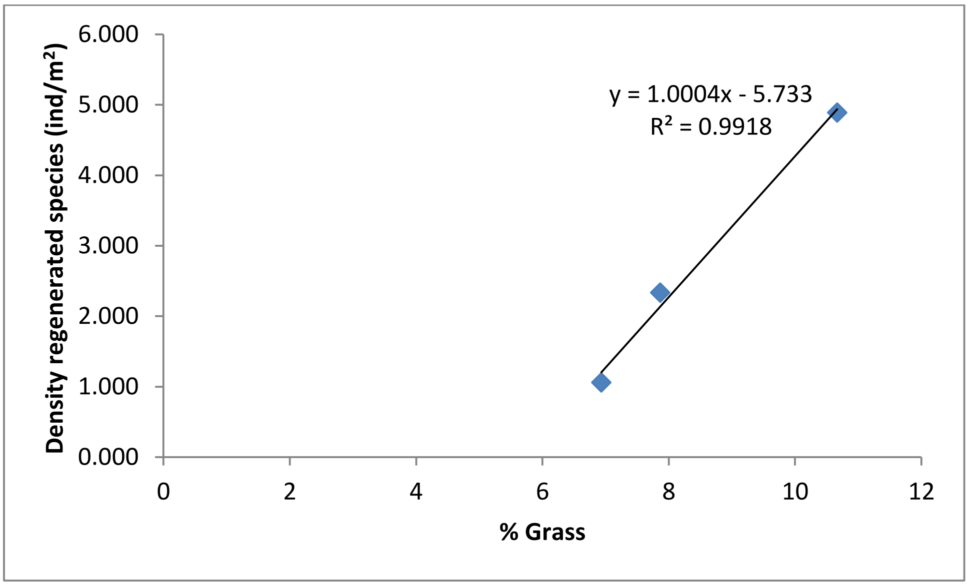

Figure 3. The relationship between grass cover and density of regenerating species for sites two, three, and four (mean, n=18).

Species Richness and Diversity

Site three had the highest alpha diversity and the highest corrected richness, and site four had the lowest. Site three had about 1.7 times higher alpha diversity and about 1.5 times higher corrected richness than site four (Figure 4).

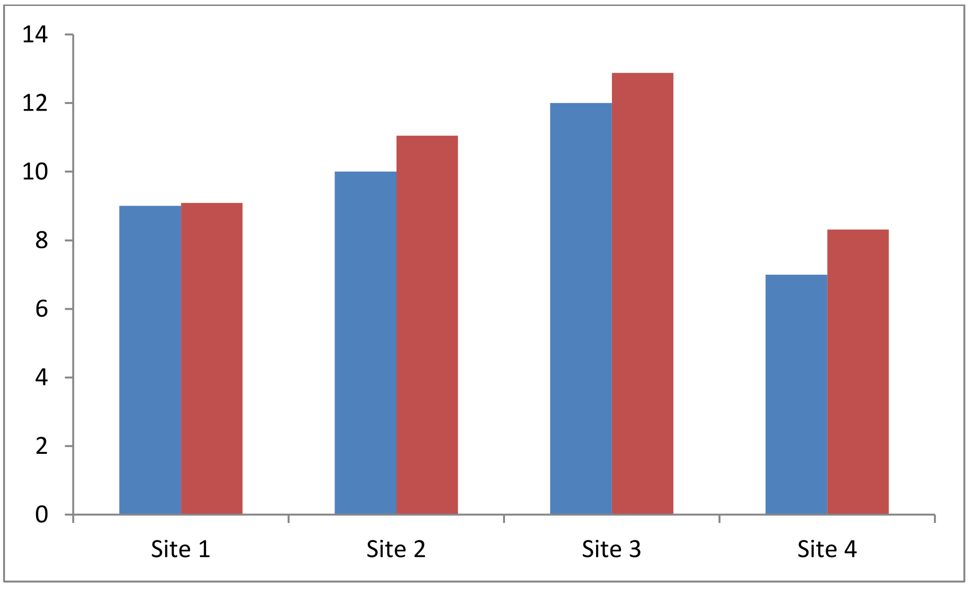

Figure 4. Alpha diversity (blue) and corrected richness (red) for each site.

Site one had the highest values for Inverse Simpson's diversity, about 2.9 times higher than site three. Site one also had the highest value for Exponential Shannon's diversity, about 2.2 times higher than site three (Figure 5; see appendix for table of richness index values for all sites).

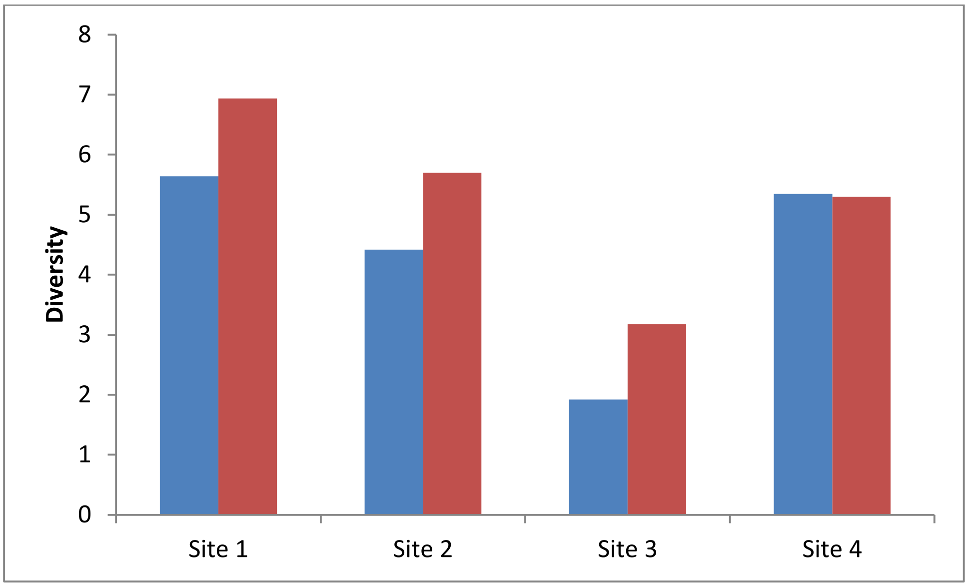

Figure 5. Inverse Simpson's (blue) and Exponential Shannon's (red) for all sites.

Beta Diversity

According to the Morisita-Horn index, site two and three were the most similar and site one and three were the least similar. The value for site two and three was about 29.2 times higher than the index for site one and three (Figure 6; see appendix for species abundances in each site and full beta index values).

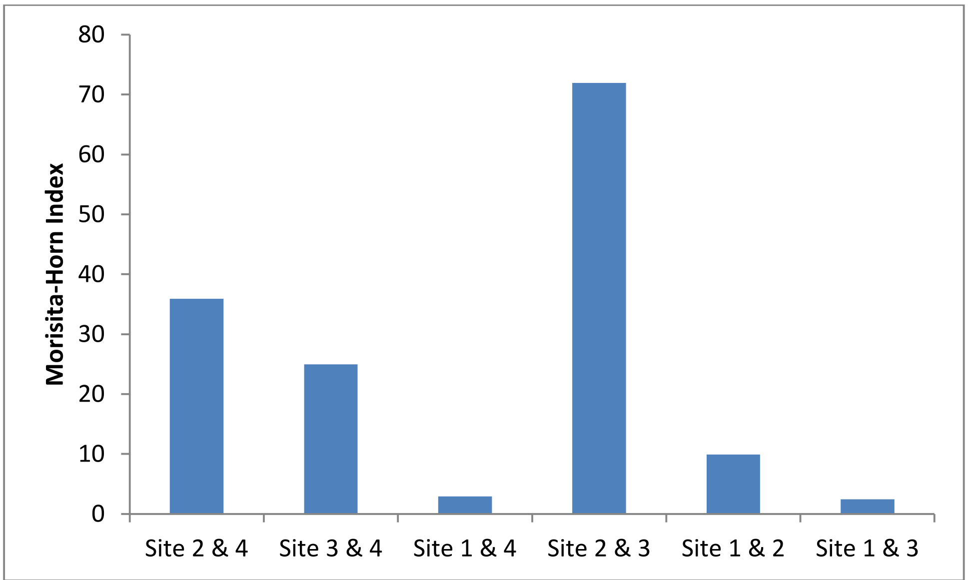

Figure 6. Morisita-Horn Index values for Beta diversity comparisons between all sites.

Important Value Index

In site one, Solano (*Solanum sp*.) had the highest importance value index (IVI), in site two and three, Ortigillo (*Urtica sp*.) had the highest IVI, and in site four, *Piperaceae* and Palmito (*Prestoea acuminate*) had the highest IVI. In site one, the majority of species were colonizers and dispersed by either animals, birds, or both. In site two, the majority of species were pioneers and dispersed by animals and birds. In site three, the majority of species were secondary species and dispersed by animals and birds. In site four, species were mainly a mix of pioneers, colonizers, and secondary species and dispersed by birds (Table 2).

Table 2. Classification, type of seed dispersal, and importance value index for all species in each site.

Study Site	Species	Classification	Seed dispersal	Importance Value Index (IVI)
One	Solano	Pioneer	Animals, birds	55.56231
	Buconia	Colonizer	Birds	25.87639
	Naranjilla	Colonizer	Animals	24.19453
	Cujaco	Colonizer	Birds	23.24215
	Platanilla	Secondary	Birds	23.24215
	Chilco	Colonizer	Wind	16.12969
	Drago	Pioneer	Wind, birds, ants	13.27254
	Cautillo	Pioneer	Animals, birds	9.24012
	Mora	Pioneer	Animals, birds	6.16008
	Mamona	Colonizer	Explosion	3.08004
Two	Ortigillo	Colonizer	Rolling, wind, birds	70.23810
	Juan negro	Pioneer	Wind	53.57143
	Palmito	Primary	Birds	16.66667
	Cautillo	Pioneer	Animals, birds	11.90476
	Solano	Pioneer	Animals, birds	11.90476
	Piperaceae	Pioneer	Animals, birds, bats	11.90476
	Teniche	Secondary	Wind	5.95238
	Lechero	Colonizer	Animals, birds	5.95238
	Buconia	Colonizer	Birds	5.95238
	Pera	*	*	5.95238
Three	Ortigillo	Colonizer	Rolling, wind, birds	120.07580
	Teniche	Secondary	Wind	18.93939
	Chupaquinde	Secondary	Birds	17.04545
	Piperaceae	Pioneer	Animals, birds, bats	10.60606
	Chilco	Colonizer	Wind	8.33333
	Platanilla	Secondary	Birds	4.16667
	Buconia	Colonizer	Birds	4.16667
	Pera	*	*	4.16667
	Mora	Pioneer	Animals, birds	4.16667
	Inga sp.	Secondary	Birds	4.16667
	Solano	Pioneer	Animals, birds	4.16667

Four	*Piperaceae*	Pioneer	Animals, birds, bats	62.82895
	Palmito	Primary	Birds	62.82895
	Ortigillo	Colonizer	Rolling, wind, birds	23.02632
	Aracaceae	Primary	Birds	16.77632
	Naranjilla	Colonizer	Animals	11.51316
	Inga sp.	Secondary	Birds	11.51316
	Chupaquinde	Secondary	Birds	11.51316

Species accumulation curves

Site one was the most complete sample, while site three was the most diverse but least complete, and site four was the least diverse (Figure 7; see appendix for species accumulation curves separated by site).

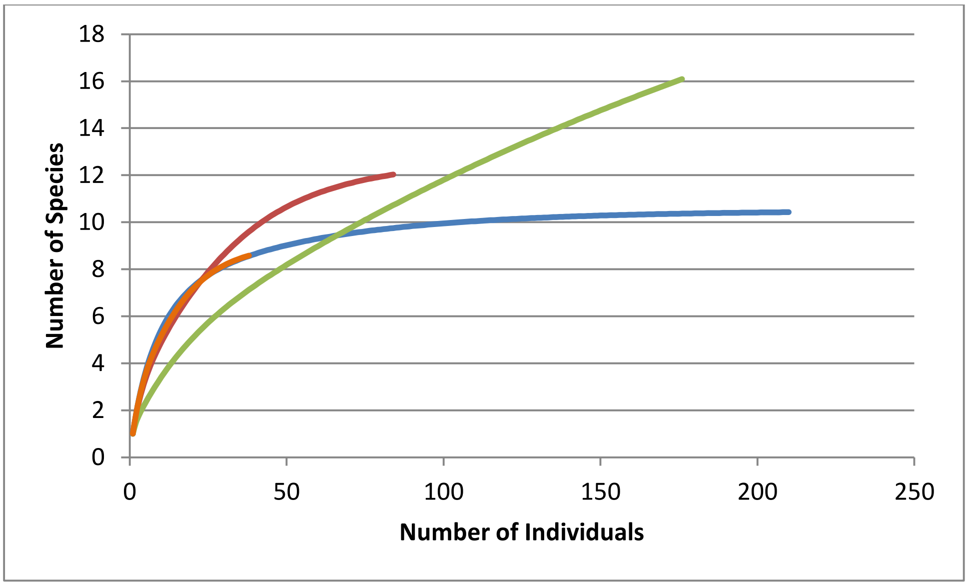

Figure 7. Species accumulation curves for site one (blue), site two (red), site three (green), and site four (orange).

Analysis of site one

Within site one, plot three had about 1.5 times as much grass cover as the control plot and plot one had about 2 times as much herb coverage as the control plot. Plot two had the highest grass and herb cover, about 1.5 times as much as the control plot. Plot one had the most *Tithonia* coverage and the control plot did not have any. Plot three had 2.1 more herb species on average than plot one. The control plot had the highest density of regenerating species, with about 2.4 times the density of plot one and plot two. Plot four had the highest number of total regenerating species, 3 more than plot one and the control plot (Table 3).

Table 3. Habitat descriptions of all plots in site one (mean, n=6 for control and n=9 for plots 1-4).

Plot	% Grass	% Herb	% Grass + Herb	% *Tithonia*	# Herb Species	Density regenerated species	Total # regenerated species
control	25.000	14.267	39.267	0.000	8.833	9.667	6
1	26.644	28.289	54.933	12.556	7.444	3.999	6
2	35.333	24.311	59.644	2.800	8.778	3.999	9
3	36.756	17.600	54.356	1.111	9.556	6.000	8
4	28.756	17.289	46.045	2.467	7.778	7.222	9

There was a significant negative correlation between the density of regenerated species and the coverage of grass and herbs in the plots in site one (correlation and linear regression, p=0.0055; Figure 8). No other significant relationships were found.

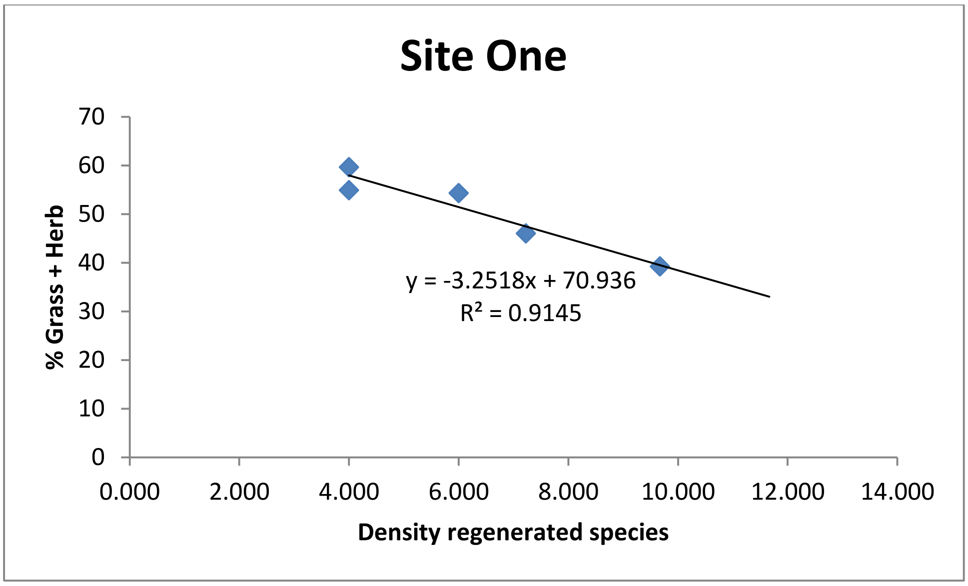

Figure 8. Density of regenerated tree species in relation to coverage of grass and herb for all plots in site one (mean, n=9).

Discussion & Conclusion

As expected, site one had the highest density and diversity of regenerating species according to Inverse Simpson's and Shannon's Indices. This result is probably because the high density and diversity of planted species facilitated the germination and growth of other species by improving the microclimate. Site one was also directly next to the forest, the closest of all sites, which increased seed input. Seed rain and diversity of seeds is high at the edge of the forest, and decreases with distance from the forest (Holl, 2006). Density and diversity of regenerating species was expected to increase with decreasing grass and herb cover. The results contradicted this prediction because there was a significant positive relationship between grass cover and density of regenerated species for sites two, three, and four. Site one had the most herb species, the highest coverage of herb and grass, and also the highest density of regenerated species. This is because site one received direct sunlight and the results showed that grass and herb cover increased significantly with the amount of sunlight. Therefore, it is likely that low seed input limits natural regeneration more than competition with invasive species. Site one was dominated by Solano (*Solanum sp.)*, a pioneer species with seeds dispersed by birds and animals. This shows that early succession is successfully taking place. It also confirms that seed dispersers are more likely to be found close to the forest edge.

Site three had the highest Alpha diversity and corrected richness but was the least complete. It had the second highest density of regenerated species and the least amount of light, which inhibited the growth of shade-intolerant invasive grasses and herbs. Low grass and herb coverage means that there is less competition for space, nutrients, and water, which improves conditions for tree species to establish. Another explanation for the high density of regenerated species is that the *Tithonia* bushes improved the soil quality by providing key nutrients such as Nitrogen and Phosphorous. Site three had the lowest Inverse Simpson's and Exponential Shannon's value, and was the furthest from the forest edge, suggesting that low seed input inhibited diverse regeneration. Sites two and three were most similar according to the Morisita-Horn Index. Both sites had low amounts of light and low grass and herb cover, and the most dominant species in both sites was Ortigillo (*Urtica sp.*), according to the Importance Value Index. This suggests that Ortigillo is a shade-tolerant species that thrives in nutrient-rich soil provided by the *Tithonia* bushes and Aliso trees. Ortigillo is a colonizer species that is dispersed by rolling, wind, and birds. As expected, wind-dispersed seeds are more likely to be found than animal-dispersed seeds far from the forest edge (Holl, 2006).

As expected, site four had the lowest density of regenerated species, lowest Alpha diversity, and lowest corrected richness. Passive regeneration in highly disturbed areas is slow and unsuccessful due to low seed rain, poor soil and microclimate conditions, and invasive species (Pedraza & Williams-Linera, 2003). Site four had the lowest grass cover and the lowest number herb species, which is most likely because of the shade provided by mature trees within and surrounding the site. This result suggests that seed rain is a more important factor for successful regeneration than competition with invasive grasses. *Piperaceae* was the most dominant species in site four, which is a pioneer species with animal, bird, and bat dispersal.

Within site one, there was a significant negative correlation between grass and herb coverage and density of regenerated species, which was expected because invasive grasses out-compete tree seedlings. However, Holl (2006) found that there was no significant difference in seed germination in pasture with grass vs. pasture with grass removed. It was found that some species actually have higher germination rates in grassy areas, so the relationship between grass coverage and seedling density is not straightforward. It was predicted that complete eradication of grass with root would lead to less coverage of grass and herb and higher density of regenerated species. Plots with complete eradication of grass and root (plots one and two) had only 2% less grass coverage, but had more herb species and overall had 7% more herb and grass coverage and almost half the density of regenerated species than the plots with superficial eradication of grass (plots three and four). Therefore, complete grass eradication was not a successful treatment in decreasing grass cover and increasing regeneration. A more successful method for decreasing grass and herb cover is to use shade, as shown in sites two and three.

The differences between the dense and diverse treatments (plots two and four) and the simple treatments (plots one and three) were as expected. The dense and diverse plots had a higher number and density of regenerated species than the simple plots. The reason for the more successful regeneration in the dense and diverse plots is that the planted tree species facilitated the growth of other species. The dense and diverse plots also had a lower coverage of grass and herbs, meaning that there was less competition. However, the results show only minor differences between the treatments, and so further monitoring should continue to confirm the success of the dense and diverse treatment. Overall, the control plot had the highest density of regenerated species and the lowest grass and herb coverage, which was not expected because it had no treatment. This result suggests that the treatments were not effective in increasing regeneration. Other factors such as high seed rain and proximity to the forest edge were probably more important. A better method for reducing grass cover should be explored.

This study showed that the natural regeneration is mostly limited by low seed rain. According to Guariguata & Ostertag (2001), seeds from seed bank more important for secondary succession than dispersed seeds. This was confirmed in the results that show that site one had the highest density of regenerated species, which had seeds and seedlings planted, and was close to the forest edge. However, it is also important to increase seed dispersers, especially in areas far from the forest edge. Mature trees attract animals by providing fruit and birds by providing branches to perch (Guariguata & Ostertag, 2001). This was true in site two, where the Aliso trees provided a perch for wild turkeys, which are known to disperse Palmito (*Prestoea acuminata*) seeds. Harsh microclimate conditions such as invasive species, drought, direct sun and poor soil nutrients are also limiting factors. Grass can out-compete tree seedlings for space both above the ground and below the ground because of large root systems (Guariguata & Ostertag, 2001). This study showed that shade was more successful than manual grass removal for reducing the cover of grass and herbs, but that ecological restoration using shade is slow. In areas dominated by invasive grasses, human intervention to decrease grass cover should be combined with high seed input to speed up natural regeneration.

There are alternative methods to encourage natural regeneration which were not analyzed in this study but are important to discuss. Cattle negatively impact natural regeneration because they damage seeds and cause soil compaction. However, minimal use of cattle can actually help regeneration. According to Griscom, Griscom, & Ashton (2009), cattle in low densities have a positive impact because they consume invasive grasses and disperse seeds in their dung. This could have been a factor in site two, where cows occasionally entered the area. Another method to encourage regeneration is to use herbicide to kill invasive grasses and herbs. However, herbicide can also damage trees by killing root systems, so this method is most effective when trees are germinating from seed rather than root (Griscom, Griscom, & Ashton, 2009). An area of study that was not included in this study is riparian zones, which have successful natural regeneration due to higher seeds dispersal by bats and birds (Griscom, Griscom, & Ashton, 2009).

This study could be expanded by also studying the different methods mentioned above. It would also be beneficial to lengthen the study to monitor long-term recovery, especially in the active experiment which is in very early succession. Plant heights should be monitored to analyse growth rates. A soil analysis for each site would be beneficial to assess how the quality of the soil affects regeneration success. The goal of ecological restoration is for the ecosystem to be self-sustainable and for the structure and functions of the ecosystem to recover. The reserve and the community should continue to monitor different methods of intervention to achieve the most successful forest regeneration.

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Appendix

Description of species

Colonizer species: the first to establish following a disturbance. They germinate in direct sunlight and are tolerant of extreme conditions such as drought and high exposure.

Buconia sp.
Papaveraceae family
The *Buconia* genus contains 10 species of soft-wooded trees, common in montane cloud forests. They grow in disturbed sites and are commonly found along roadsides. The leaves are feather-like with lobes rising at multiple points from common axis. The inflorescence is a large panicle, with multiple branches forming off a main axis. The fruits have a spindle-like shape, wide in the middle and tapered at both ends, and are orange or salmon in color.
Seed dispersal: birds.

“Camacho”: *Xanthosoma sp.*
Araceae family
The genus contains 50 species native to tropical America. They are monocot herbs, growing best in partial shade and moist soil. The simple, alternate leaves are 40-200cm long and are palmate, growing on long leaf stalks. They are known as “elephant ear” because of the heart-shaped leaves. The flowers are white and pink in a spathe. The fruits are berries. Many species are a starchy food staple or used as ornamental plants.
Seed dispersal: small rodents, beetles

“Chilco”: *Baccharis sp*.
Asteraceae family
Chilco is a floweing plant common in South America and endemic to the South of Brasil, Paraguay, Uruguay, and Northeast Argentina. It can grow up to 2m and establishes in open areas. Flowers are white or pink and usually disk-shaped. Leaves are small and thin and have 3 main veins. There are more than 500 species in the *Baccharis* genus. A few species are nearly extinct in the Northern Andes due to habitat destruction. Many species are used in folk medicine to treat stomach, liver, and prostate diseases. Some butterfly and moth species feed on this plant and it is host to fruit fly larvae. The fruit type is achene and contains only one seed.
Seed dispersal: wind

“Cujaco”: *Solanum cucullatum*
Solanaceae family
The distribution is in premontane or montane humid forest in the Western Andes of Ecuador and Peru, at an elevation of 700-2500m. The flowers are white, lavender, pink, or blue with yellow centres and 5 petals. The leaves form in pairs and are triangular with gentle lobes or toothed. The fruits are small green or black balls, poisonous to humans.
Seed dispersal: birds

“Guayaba”: *Psidium guajava*
Myrtaceae family
Weedy trees, native and cultivated in Ecuador. It tolerates drought and high temperatures, and grows in a wide range of soils that are heavy clay to light sand. The leaves are opposite and can be eaten to stop diarrhea. The fruits are edible and high in ascorbic acid, and can also be used as anti-diarrheal. The bark is thin and peels. The flowers have five petals. This plant is invasive in the Galapagos Islands.
Seed dispersal: animals

“Lechero”: *Sapium glandulosum*
Family: *Euphorbiaceae*
Native to the neotropics from Mexica to Argentina. It is a woody tree that can grow up to 30m tall and has buttress roots. It grows in humid montane forests up to an altitude of 3200m. The bark is thin and the surface is covered in short, thick spines. The fruit is a green/brown rounded capsule with 3 segments, each segment containing one seed. The seeds are covered in a thin red pulp. The leaves are oval with toothed edges and can be up to 27cm long. The

inflorescence is spikey, formed from a cluster of male flowers with female flowers at the base with 3 styles. It has glands that produce a milky latex that is used to make rubber. It is listed by the IUCN as Vulnerable.
Seed dispersal: birds and animals

"Mamona": *Ricinus communis*
Euphorbiaceae family
The *Ricinus* genus only contains one species. It is a fast-growing flowering shrub that can reach 12m in height. It is native to the Mediterranean basin and Eastern Africa, and is widespread throughout the tropics. It is introduced and cultivated in Ecuador. The leaves are palmate with gradually tapering and serrated lobes. It cannot live in cold temperatures, but is tolerant of a wide range of soil types, and can germinate in direct sunlight. This makes it a competitive plant that is indicative of a disturbed area and commonly establishes in waste areas. The seeds are poisonous to humans, insects, and many animals. It is known as the "castor oil plant" because the bean produces an oil that is used in the manufacturing of soaps and lubricants. The oil can also be used for medicinal purposes to treat warts, tumors, and indurations (hardening) of the abdominal organs. The oil is also a laxative.
Seed dispersal: explosion

Miconia sp.
Melastomataceae family
The *Miconia* genus contains 1000 species, making it one of the largest neotropical plant genera. There are 258 species in Ecuador, of which 97 are endemic. Most species are shrubs or small trees, with a few large or emergent trees, growing in moist forests. Some species have stilt roots and papery bark with red sap. The leaves are white or tan on the underside and hairy. The terminal inflorescence is a large panicle, with broad small petals and white or light pink blossoms. The fruits are purple berries containing hundreds of tiny seeds and are sweet to attract dispersers, such as deer, squirrels, and guans. Each plant can produce hundreds of thousands of seeds that can travel long distances, making it aggressive and invasive, sometimes a threat. The seeds can lie dormant for many years and germinate in sunlight. The root system is shallow and combats soil erosion.
Seed dispersal: birds and animals

"Mora": *Rubus sp*
Rosaceae family
21 species in Ecuador, including three endemic. Small shrubs with spiny stems and compound leaves. Fruits are berries and are edible. The shrub is aggressive and invasive in certain parts of Ecuador, including the Galapagos.
Seed dispersal: birds and animals

"Naranjilla": *Solanum quitoensis*
Solanaceae family
Naranjilla is a small flowering shrub that is cultivated for fruit but also grows in secondary forests. It is native to Ecuador. It grows best in partial shade, protected from strong winds, and is not adapted to full sun or high temperatures. Grows at altitudes between 1500-1800m.

Can grow up to 8ft high, producing little orange citrus fruits that are highly acidic. Leaves are alternate and oval-shaped, with the leaves and stem covered in purple hairs. Indigenous and most abundant in Peru, Ecuador, and southern Colombia. Can grow on a variety of soil types including rocky soil with poor nutrients. It is widely cultivated in Ecuador for the fruit that can be eaten raw or made into juice.
Seed dispersal: animals

"Ortigillo": *Urtica sp.*
Urticaceae family: contains mostly herbs, sometimes shrubs or small trees
The *Urtica* genus contains 50 species of herbaceous plants. They grow at high altitudes and are common in the Andes. They are also found in North America, Europe, Asia, and Northern Africa. They grow best in humid soils high in Nitrogen. The leaves are opposite with serrated edges, 3 veins, and stinging hairs that cause skin irritation and protect against herbivory. The inflorescence is axillary, with small green unisex flowers in short spikes. The plants provide food for many butterflies, and provide shelter for aphids, caterpillars, and moths, which are fed on by birds.
Seed dispersal: rolling, wind, and birds

*Tithonia diversifolia: "*Girasol del monte"
Asteraceae family
Shrub native to Mexico and Central America, introduced to Ecuador. It has moderate drought tolerance and grows quickly, commonly planted as a hedge or along roadsides. It grows up to 6m and has large yellow or orange flowers. It is often used in agriculture as a natural fertilizer because it raises soil fertility and adds nutrients such as Nitrogen, Phosphorous, and Potassium to the soil.
Seed dispersal: Wind, water, or animal

Pioneer species: establish soon after a disturbance, indicating early secondary succession. They generally having the following characteristics: rapid growth, low density wood, many seeds that are easily dispersed, require direct sunlight to germinate, and a short lifespan of 7-25 years (Corlett, R. T., 1995). They are tolerant of extreme conditions, such as wind and drought. These species increase the fertility of the soil with Nitrogen and organic matter, and provide shade, thereby facilitating the growth of future successional species (Finegan, B., 1984).

"Aliso/Alder": *Alnus nepalensis* and *Alnus acuminata*
Betulaceae family – common trees of secondary montane forests.
The *Alnus* genus contains three species in the Central America and the Northern Andes, and 30 species in the USA and the Old World. They are flowering, deciduous, medium-sized trees, with simple, alternate, leaves that are small and round with serrated edges. They commonly grow near streams and rivers. The flowers are catkins, with male flowers longer than female flowers, pollinated by the wind. The bark is thin and grey and often covered in white lichen. The fuits are small brown cones that contain many tiny seeds. Alder trees are capable of establishing in poor soils because they improves soil fertility by adding key

nutrients. The roots have a symbiotic relationship with bacteria, *Frankia alni*, that live in root nodules and allow the tree to fix nitrogen. Used for erosion control and timber harvested for firewood.
Seed dispersal: wind

"Cautillo": *Sapium laurifolium*
Euphorbiaceae family
The *Euphorbiaceae* family has 300 genera with around 8000 species The Sapium genus comprises 125 species. Cautillo is a perennial flowering plant native to Cuba, Haiti, Jamaica, Venezuela, Brazil, Colombia, Ecuador, and Peru. The Sapium genus contains 95 species in the New World and 25 species in the Old World. The large trees are common and indicate secondary succession. The tree produces latex and has a pair of glands below the petiole apex. It has a spike-like inflorescence, with both male and female flowers with two to four stamens and styles and no petals. The fruit has two to four lobes. The leaves are alternate with a finely serrated edge. Historically it has been cultivated for oil to produce soap.
Seed dispersal: animals and birds

Clusia sp.
Clusiaceae family
The *Clusia* genus contains 300-400 species native to tropical America. There are 63 species in Ecuador and 5 are endemic. They comprise shrubs, vines, epiphytes, and trees which can grow up to 20m tall. They have large, leathery, opposite leaves up to 30cm long. The flowers are white, cream, yellow, pink, red, blackish or green with 4-9 petals. The fruit is a capsule that contains several seeds, which are covered in a fleshy coat. Some species used as incense.
Seed dispersal: birds and animals

"Cordoncillo": *Piper sp.*
Piperaceae family: shrubs, herbs, climbers, and some trees with stilt roots; 13 genera, 3600 species.
The *Piper* genus contains 2000 species, the majority of which are shrubs with swollen nodes and a peppery odor. There are 233 species in Ecuador, including 75 endemic. They can be terrestrial or epiphytic. Leaves are simple, located along the stem of at the base of the plant. The spikey inflorescence contains unisex flowers and is located opposite the leaf. The fruit is a berry or drupe containing one seed. Roots can be used to treat internal haemorrhaging. The essential oil is anti-bacterial and anti-fungal, and repels pests of cattle. The leaves are anti-inflammatory and used for stomach ulcers.
Seed dispersal: animals, birds, bats

"Drago": *Croton lechleri*
Euphorbiaceae family
The *Croton* genus is large and diverse, with 400 species in the New World and 350 species in the Old World. There are 48 species in Ecuador including 15 endemic. The full common name for this tree is "sangre de drago," and it is native to northwest South America. The tree is medium to large, growing up to 35m tall, and common in disturbed areas. The inflorescence is a spike of small white flowers, and the fruit is also a spike, containing three

seeds. The tree produces a thick red latex known as “dragon’s blood” that has important medicinal uses. It can be used as a liquid bandage, and to treat diarrhea, inflammation, wounds, tumors, stomach ulcers, herpes infection, and insect bites.
Seed dispersal: birds, wind, and ants

“Espino santo”: *Berberis sp*.
Berberidaceae Family: 15 genera, about 570 species
The *Berberis* genus contains 450 species, mostly in the Old World. They are high altitude shrubs and small trees, 1-5m tall. They grow well in shaded areas and disturbed areas. The leaves are often spiny and clustered on bracteate short roots. Most species have trifurcate spines which are unique to this family. The flowers are yellow or orange with 6 petals and 6 sepals. The fruits are blue and rounded or spindle-shaped, with an acidic taste. They can be eaten raw and used to make marmalades and jams. They are also used as ornamental plants or hedges.
Seed dispersal: birds and small mammals

“Juan negro”: *Piptocoma discolour*
Asteraceae family
This flowering tree is native to the Costa Rica, Panama, and the Amazon region of Ecuador, Peru, and Columbia. It can grow to a height of 30m and a diameter of 60cm. It often grows in disturbed forest undergoing early or late succession. It is abundant and resilient, and grows naturally in fallows and soils with clay and silt. The soft wood is harvested to make boxes to transport organic produce. For this reason it is used in sustainable management and is an important source of income to rural families.
Seed dispersal: wind

“Solano” or “tomatillo”: *Solanum sp.*
Solanaceae family
Solanum is the biggest genus in the *Solanaceae* family, containing 800 species in the New World and 600 species in the Old World. There are 183 species in Ecuador, including 33 endemic. Most of the species are small trees, some are large trees with spiny trunks, some are herbs, and a few are soft-wooded shrubs. The spiny leaves are alternate, pinnately compound, and aromatic.
Seed dispersal: birds and animals

Secondary species: Secondary species indicate a forest in medium to late succession. They are generally more slow-growing and have a longer life-span and fewer seeds than coloniser or pioneer species.

“Cascarilla”: *Cinchona pubescens*
Rubiaceae family
Native to the West Indies and naturalised to Mexico, Columbia, Ecuador, and the Amazon basin region. It is a small tree or shrub that can grow up to 20ft tall. The leaves have a metallic-silver-bronze underside, and are white-ish on top. The flowers are small with white petals and strong fragrance. The bark is scented with a pale yellow-brown color and has

medicinal uses. The seeds are winged so they can travel in the wind, and they germinate in sunlight but seedlings are shade-tolerant. It can be used as a tonic to reduce fever, aid digestion, lower blood pressure, or as a mild stimulant. It has become an invasive species in many tropical areas including the Galapagos Islands.
Seed dispersal: wind.

"Chupaquinde": *Palicourea sp.*
Rubiaceae family
The *Palicourea* genus contains 200 species of shrubs and small trees and is the most dominant genus of *Rubiaceae* in montane forests. There are 80 species in Ecuador including 22 endemic. The leaves are leathery with smooth edges, and are occasionally lobed. The fruits are berry-like with one or two seeds. The yellow or purple corolla is tubular and typically around 7mm with basal swelling. The ovary is inferior.
Seed dispersal: birds

Ficus sp.
Moraceae family: one of the most important neotropical tree families, especially on fertile soils
The *Ficus* genus contains 150 species in the New World and 600 species in the Old World. There are 56 species in Ecuador including two endemic. It is the largest genus of the *Moraceae* family and majority of the species are strangler figs. They produce white latex. The terminal stipule is conical and well developed. The leaves have close-together secondary veins that branch at a wide angle from the primary vein. The fruit is a hollow round structure with the inner surface lined with tiny flowers or fruitlets. It is pollinated by specific species of wasps that have a mutualistic relationship with the fig tree and lay eggs inside the fig fruit.
Seed dispersal: animals, for example bats and monkeys, and birds.

"Guaba": *Inga sp.*
Fabaceae family, *Mimosoideae* subfamily – usually trees, some vines and lianas.
The *Inga* genus contains 300 species, many native to the Amazon region. Ecuador has 81 species including 10 endemic. Some species are Nitrogen-fixing large trees and shrubs. The leaves are compound and pinnate, with cup-shaped glands between each leaflet pair. Nectar on the leaves attracts ants. The flowers are pollinated by hummingbirds and the fruit eaten by parrots. The seeds come in pods up to 1m long and are covered with sweet white powder and a pulp that can be eaten raw. Medicinal uses include treatment for bronchitis and wounds.
Seed dispersal: birds

"Huevo del mono": *Posoqueria sp*.
Rubiaceae family: one of the largest and most prevalent neotropical families. Very easy to recognise by simple, opposite leaves with interpetiolar stipules.
The *Posoqueria* genus contains 15 species of midcanopy trees that grow in wet topical forest. They are native from to the Americas from Mexico to Northern South America. They are fast growing, with a height of 8-20ft. They grow best in rich, moist soils and have a low drought tolerance. The leaves are simple and opposite. The flowers are long, white, fragrant tubes, pollinated by hawkmoths, that can grow up to eight inches long and have 5 petals. The fruit is

a yellow woody berry, about two inches wide, containing one seed surrounded by pulp, that can be eaten raw.
Seed dispersal: animals, especially monkeys

“Moquillo”: *Saurauia sp.*
Actinidiaceae family
The *Saurauia* genus is the only neotropical genus of this family and contains 80 species in the neotropics and 160 species in tropical Asia. The trees grow in mid-elevation forests and are often weedy, growing 5-10m tall. It can grow in full sun or partial shade, and prefers cool tropical climates. The leaves have close-together veins, parallel secondary veins, serrate margins, and a rough surface. The fruit is a green-ish berry with many tiny seeds and sweet pulp which can be eaten raw. The flowers are white and grow in clusters of a dozen or more.
Seed dispersal: rain and birds

“Platanilla”: *Heliconia sp.*
Heliconiaceae family
The *Heliconia* genus has more than 100 species in Indonesia, Pacific islands, and tropical Central and South America. There are 65 species in Ecuador and 18 are endemic. The large herb is a monocot that is usually 1-2m tall but can grow up to 7m tall. It prefers high humidity and temperatures, and grows well in partly shaded areas with rich organic soil. The fruits are drupes with blue or purple seeds. The leaves are simple and alternate or opposite. The inflorescence is hermaphrodite, held in large bracts that are red, green, yellow, or orange and pollinated by hummingbirds. The bracts trap rain water that is a microhabitat for many invertebrates. It is valuable in reforestation because it protects water sources. It is widely used as an ornamental plant, and the leaves are sometimes used in cooking.
Seed dispersal: birds

Primary species: Primary species indicate an old-growth forest in late succession. They grow much taller than the previous tree species and come to dominate the canopy. They have long life-spans of 50-100 years. They have dense wood and often have wind-dispersed seeds (Corlett, R. T., 1995).

“Canelo”: *Ocotea sp.*
Lauraceae family: 50 genera with over 3000 species of flowering plants worldwide, native to tropical South America and Southeast Asia. The fine timber is harvested.
The Ocotea genus contains 350 species in the New World, plus a few in Africa and about 20 in Madagascar. There are 48 species in Ecuador including 6 endemic. It is the main genus in the neotropics, especially in the Amazon. Canelo can grow 4-10m tall and has grey bark. The leaves are simple and alternate. The fruit is dark green and darkens with maturity, and looks like an acorn with a single seed wrapped in a hard coat. The flowers are white and radially symmetrical. The plant is aromatic.
Seed dispersal: birds

“Palmito”: *Prestoea acuminat.*
Aracaceae family

The palm family contains 202 genera with 2600 species. Palms occupy a wide range of habitats, from desert to tropical forest. They have a diverse morphology, and include shrubs, trees, and vines. The leaves are palmately compound (fan-leaved) or pinnately compound (feather-leaved) and have parallel veins. The inflorescence is unisex or bisexual, with small white flowers, with the fertile part of the flower enclosed in bud by spathes. Ecuador has 15 species from the Bactris genus, including one endemic.
Seed dispersed: birds, especially wild turkey

Unidentified species:
Pera
Teniche (secondary species, wind dispersal)

Tables, graphs, maps, and designs

Table 3. Richness index values for all sites.

Study Site	Simpson's Index	Inverse Simpson's	Shannon's Index	Exponential Shannon's	Evenness
One	0.177	5.640	1.937	6.936	0.841
Two	0.226	4.415	1.740	5.70	0.756
Three	0.521	1.918	1.156	3.176	0.482
Four	0.187	5.344	1.667	5.296	0.857

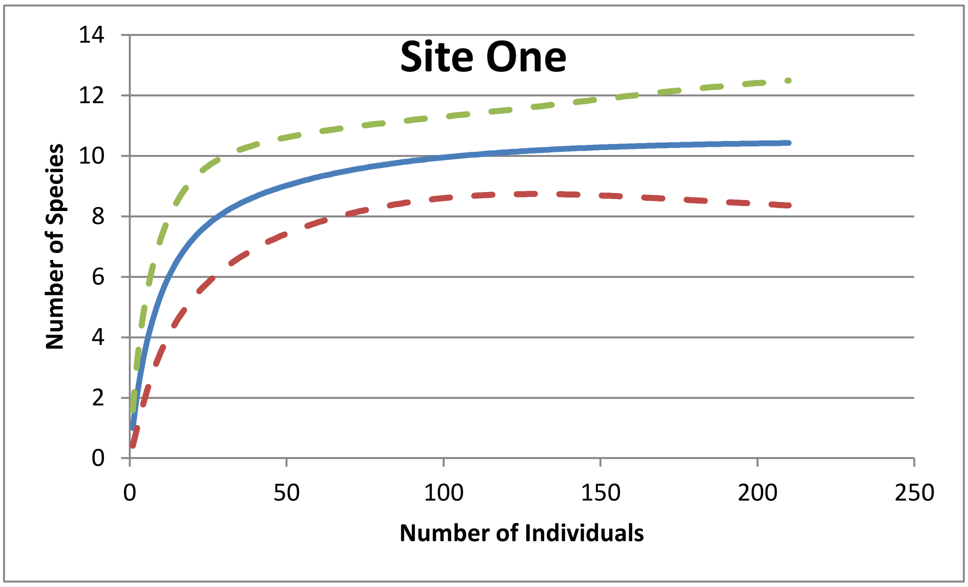
Table 4. Species abundances in each site.

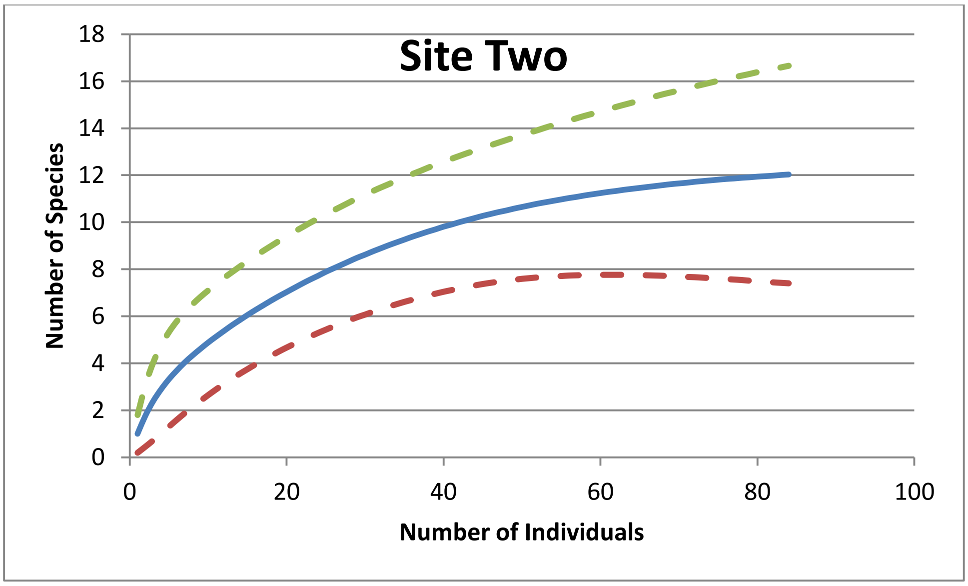
Species	Site one	Site two	Site three	Site four
Naranjilla	12	0	0	1
Piperaceae	0	2	4	6
Ortigillo	0	16	63	2
Palmito	0	4	0	6
Inga sp.	0	0	1	1
Chupaquinde	0	0	7	1
Aracaceae	0	0	0	2
Cautillo	3	2	0	0
Solano	36	2	1	0
Juan Negro	0	12	0	0
Teniche	0	1	6	0
Lechero	0	1	0	0
Buconia	16	1	1	0
Pera	0	1	1	0
Platanilla	11	0	1	0
Chilco	8	0	2	0
Mora	2	0	1	0
Kujaco	11	0	0	0
Drago	5	0	0	0
Mamona	1	0	0	0
Total	105	42	88	19

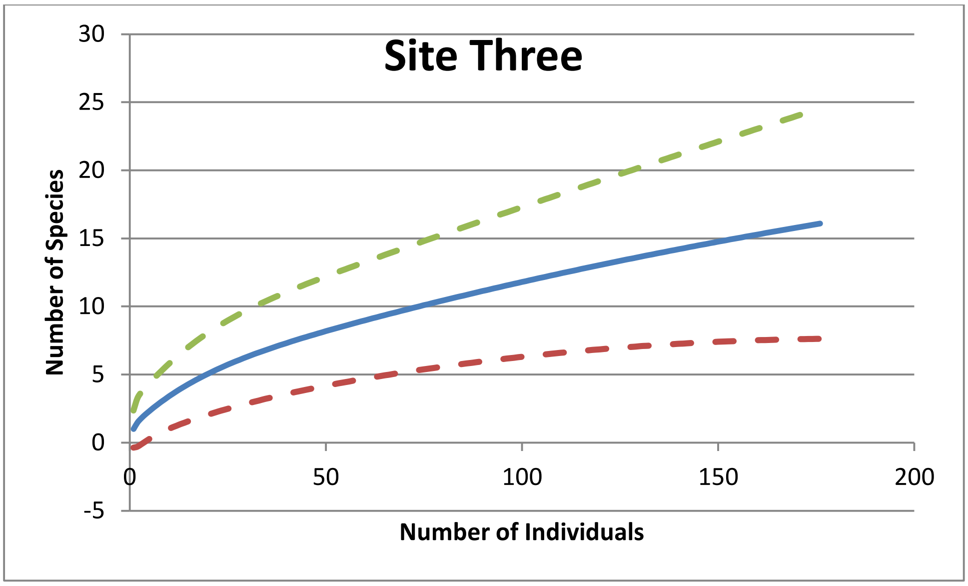
Table 5. Beta Diversity Index values.

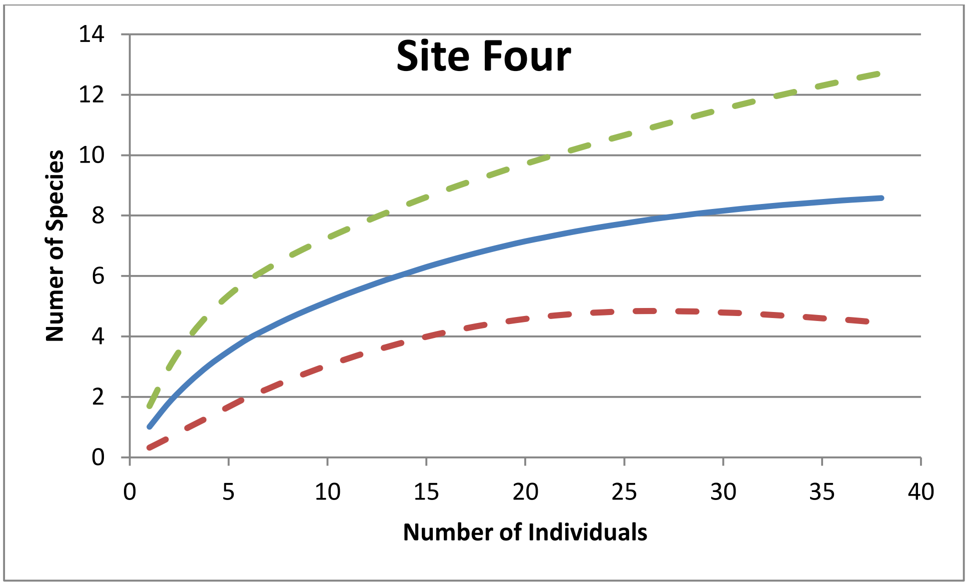
Study sites	Sorenson's Index	Jaccard's Index	Morisita-Horn Index
Two and four	35.2941	21.4286	35.8932
Three and four	44.4444	28.5714	24.9734
One and four	11.7647	6.2500	2.8985
Two and three	47.6190	31.2500	71.9390
One and two	30.0000	17.6471	9.9135
One and three	47.6190	31.2500	2.4625

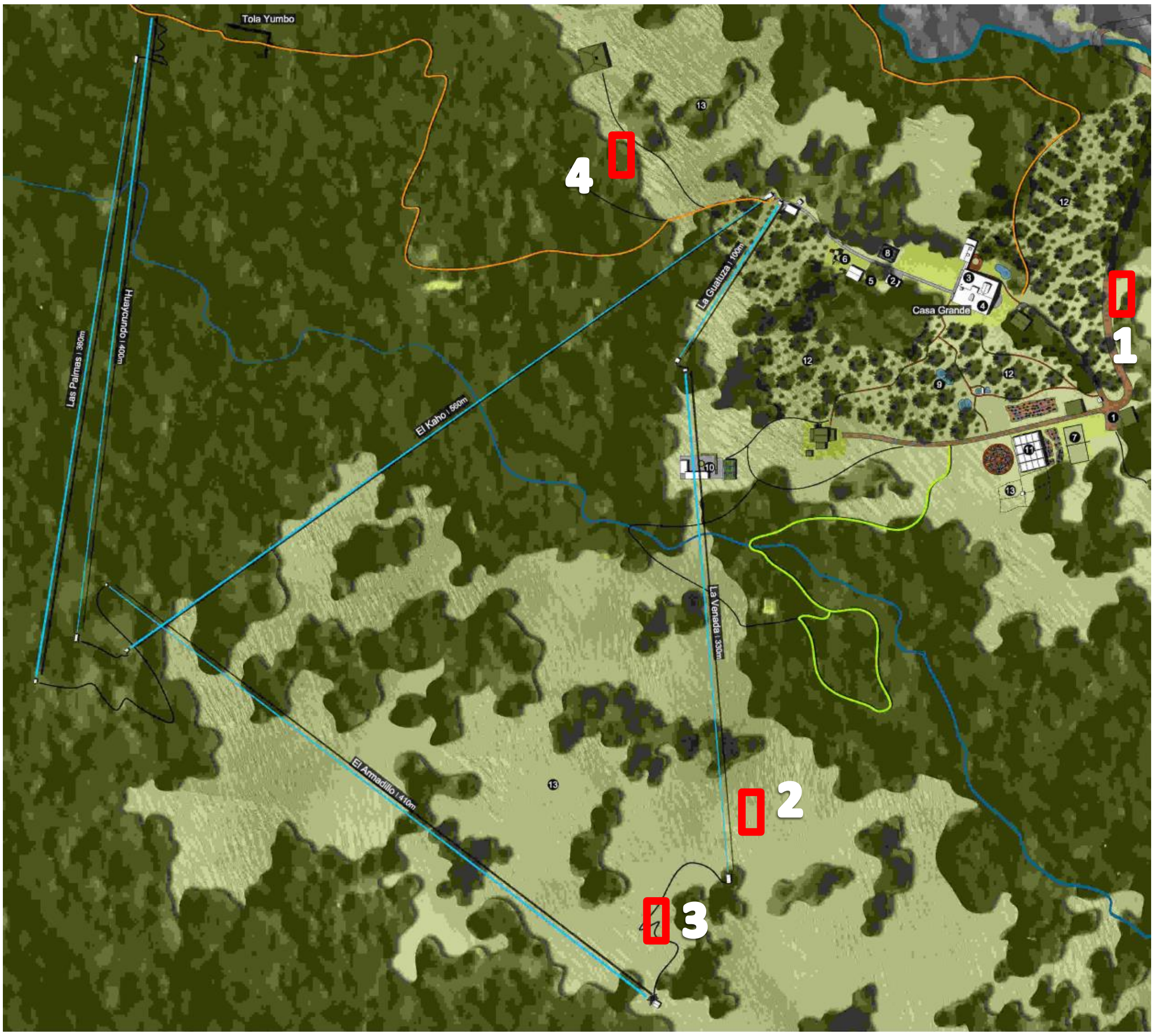
Species accumulation curves with upper and lower bounds, separated for each site.



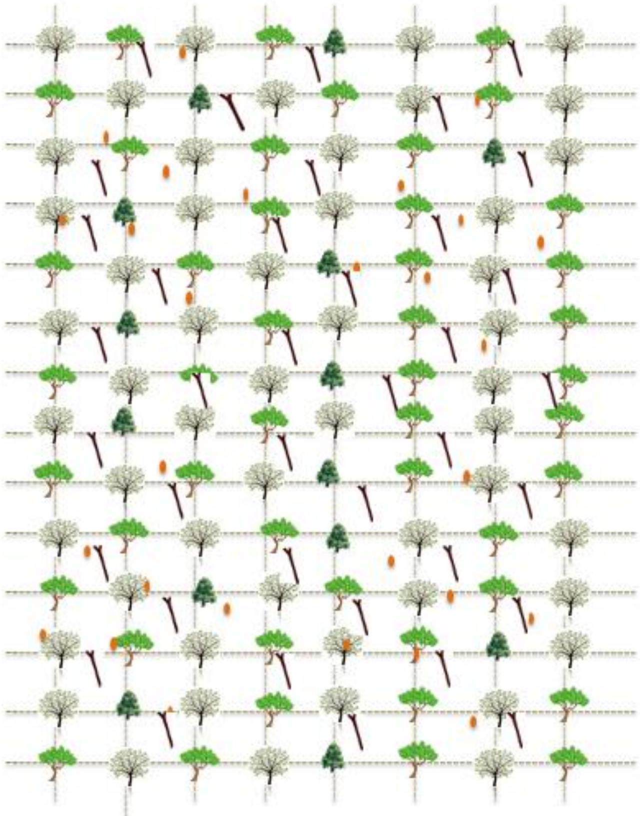


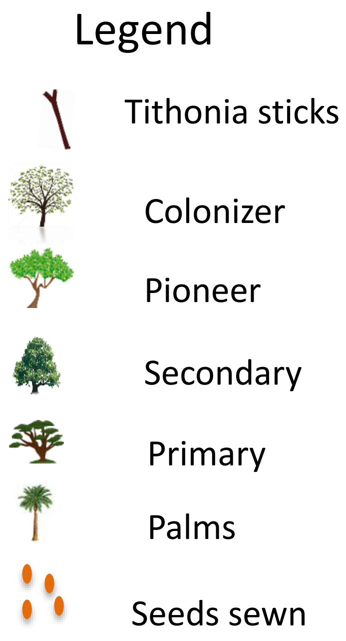

Site Three
Number of Species
30
25
20
15
10
5
0
-5
0
50
100
150
200
Number of Individuals


Site Four
Numer of Species
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
Number of Individuals

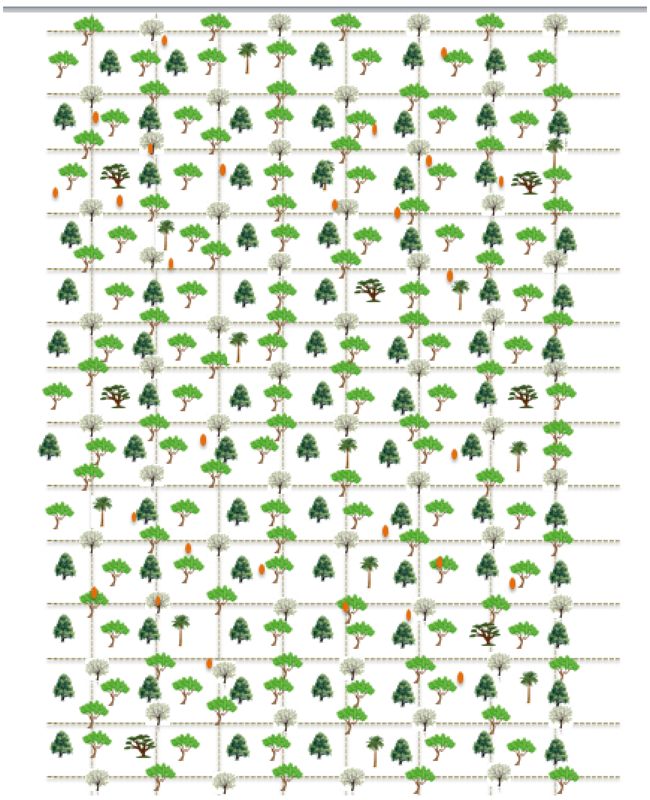


Map of Inti Llacta Reserve with study sites in red.





Design of simple plots in site one



Design of dense and diverse plots in site one